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Characterization and identification of mannanolytic actinomycete *Nonomuraea* sp. ID06-379

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ABSTRACT

Aims: The study focused on screening, identification and characterization of mannanolytic actinomycetes isolated from soil and leaf litter samples obtained from several sites in Indonesia.
Methodology and results: A total of 337 isolates of actinomycetes isolated from soil and leaf litter samples collected from various areas in Indonesia were screened for their mannanolytic activity. Mannanase activity was analysed using *locus bean gum* (LBG) as the substrate. The strain ID06-0379 displayed significant mannanase activity. The strain ID06-0379 was analysed for its mannanase activity by determining the rate of enzyme production when cultured in the presence of palm kernel cake (PKC) as a substrate. The highest mannanase activity from ID06-0379 was 4.40 U/mL at 5% PKC concentration at 5 days incubation. Chemotaxonomic and phenotypic characterisation of mannanolytic actinomycete was done and the strain ID06-0379 contained *meso*-diaminopimelic acid, and madurose was the diagnostic sugar in whole cell sugar. The polar lipids were phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylinositol, and hydroxy-phosphatidylethanolamine. The predominant menaquinone of strain ID06-0379 was MK-9(H_4). The major cellular fatty acids were $C_{16:0}$ (31.47%), cis9-$C_{16:1}$ (15.23%) and iso-$C_{16:0}$ (10.84%), and the G+C content of the DNA was 71.7 mol%. Phylogenetic analysis based on the 16S rDNA sequences revealed that strain ID06-379 was closely related to species of *Nonomuraea jabiensis* A4036T with 99% nucleotide similarity.
Conclusion, significance and impact study: The results from this study revealed that the mannanolytic actinomycete strain ID06-379 belongs to the genus *Nonomuraea* that closely related to *N. jabiensis* A4036^T. Mannanase production using agricultural waste such as palm kernel cake may contribute to the development and utilisation of biomass bioconversion processes.

Keywords: Indonesian actinomycetes, mannanase enzyme, *locus bean gum*, palm kernel cake, *Nonomuraea* sp. ID06-0379.

INTRODUCTION

Plant cell walls consist of complex polymers which are mainly cellulose, hemicellulose and lignin. Hemicellulose is the second major component of the plant cell wall and the most abundant and renewable carbohydrate consists mainly of mannan and xylan (Lynd *et al*., 2002). Hemicellulose is a matrix polysaccharide that constitutes up to 50% of lignocellulosic biomass depending on the source. Lignocellulose degradation in the natural environment is largely attributed to fungi, and the importance of actinomycetes in this process may be underestimated. The production of β-mannanase by microorganisms is more promising due to its low cost, high production rate and readily controlled conditions (Meenakshi *et al*., 2010).

Microbial mannanases are the primary endo-type enzymes responsible for degradation of mannan polysaccharides (Kim *et al*., 2011). Mannanases have been isolated from *Aspergillus* spp. (Azis *et al*., 2008), *Penicilliumfreii* (Wang *et al*., 2012), *Chaetomium* sp. (Katrolia *et al*., 2012), *Bacillus pumilus*, *Caldicellosiruptor saccharolyticus* (Frangos *et al*., 1999); *Cellulosimicrobium* sp. (Kim *et al*., 2011) and *B. subtilis* (Bo *et al*., 2009). *Thermomonospora fusca* was also reported to secrete thermostable β-mannanase at temperature of 80 °C, which hydrolyses the O-glycosidic bonds in mannan, and

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has potential use in pulp and paper production (Béki *et al*., 2003).

It is well-known that the cellulolytic actinomycetes include members of the genera *Cellulomonas* and that *Themomonospora fusca* can also produce mannanase enzymes (Béki *et al*., 2003). In the genus *Streptomyces*, mannan-degrading activity has been found in some strains (Yopi *et al*., 2006; Shi *et al*., 2011; Kumagai *et al*., 2012). Degradation of mannan is a key step in the production of bioethanol from palm kernel cake (PKC) material, as mannan constitutes 78% of PKC biomass (Wong *et al*., 2011). Structurally, mannan is composed of mannose backbone that is linked to various sugar moieties, such as galactose. Actinomycetes genera such as *Streptomyces*, *Nocardia*, *Arthrobacter* and *Rhodococus* produce hemicellulases, cellulases and lignases, all of which play an important role in lignocellulose degradation (Khanderparker *et al*., 2008). The hydrolysis of hemicellulose by hemicellulases is important in biofuel production as this polysaccharide is widely distributed throughout the lignocellulosic structure and contains a large amount of fermentable sugars.

In this study, we screened actinomycetes from our existing collection for their capability to produce mannanase enzyme. The actinomycete was isolated from rhizosphere soil and leaf litter samples obtained from several sites in Indonesia (Widyastuti and Ando, 2009; Lisdiyanti *et al*., 2012). Evaluation of the production of mannanase enzyme from mannanolytic actinomycetes using palm kernel cake as a substrate was also performed. This study may contribute to the development and utilisation of biomass bioconversion processes.

MATERIALS AND METHODS

Actinomycetes isolates

A total of 337 actinomycetes isolates collected from Purwodadi Botanical Garden, Bukit Sari Botanical Garden Jambi, Cibinong in West Java, Enrekang in South Sulawesi, Timor East Nusa Tenggara, Kutai National Park in East Kalimantan, Gili and Kuta in Lombok Island, Sungai Wain Botanical Garden, Baturraden Botanical Garden, Bantimurung South Sulawesi, and Papua, Indonesia were used in this study. The actinomycetes were isolated from rhizosphere soil and leaf litter samples and all the isolates were identified based on 16S rDNA sequences (Widyastuti and Ando, 2009).

Selection of mannanase-producing actinomycetes

All 337 isolates were cultured on *Locus Bean Gum* (LBG agar) medium containing 0.4% yeast extract, 0.4% malt extract, and 0.5% LBG (pH 7.0). Isolates were cultured onto LBG agar plates and incubated for 4 days at 28 °C. Plates were then flooded with 0.1% Congo red for 15-20 min, then washed with 1mM NaCl, and kept overnight at 5 °C. Bacterial colonies exhibiting clear zones against red colour of non-hydrolysed media were considered mannanase producers (Carder, 1986).

Mannanase production was indicated by the appearance of a pale halo with orange edges, indicative of areas of hydrolysis. This halo was measured for the subsequent calculation of the enzymatic clear zone index (CZI) using the equation:

$$CZI = \frac{\text{diameter of hydrolysis zone}}{\text{diameter of colony}}$$

Mannanase activity assay

β-Mannanase activity of strain ID06-379 was determined by monitoring the release of reducing sugars. Mannanase activity was measured following the dinitrosalicylic acid (DNS) method of Miller (1959). Briefly, a reaction mixture composed of 0.1 mL of crude enzyme solution plus 0.9 mL of 0.5% LBG in 50 mM sodium citrate buffer (pH 6.0) was incubated at 50 °C in a water bath for 30 min. The reaction was terminated by adding 2 mL of DNS reagent. The colour was then developed by boiling the mixture for 15 min. Optical densities of samples were measured at 540 nm against a blank containing all the reagents minus the crude enzyme. Results were interpreted in terms of enzyme activity in which one unit (U) of enzyme activity was defined as the amount of enzyme which liberates 1 µmol of glucose per minute under the given assay conditions (Miller, 1959).

Extracellular enzyme production

The strain ID06-379 was grown in the medium for mannanase production which contained 0.4% yeast extract, 1% malt extract, and 0.4% locust bean gum (pH 7.3). A 250 mL flask containing 50 mL of the medium was inoculated with a loopful of cells taken from a stock slant and was pre-cultured at room temperature on a shaker (120 rpm) for 3 days. The same volume of medium and flasks for enzyme production were inoculated with 5 mL of the culture and cultivated at room temperature for 8 days. Aliquots of the culture medium were sampled at 1 day intervals to determine β-mannanase activity.

Enzyme production under submerged fermentation with palm kernel cake as substrate

The strain ID06-379 was grown under submerged fermentation by taking 25 mL of submerged fermentation medium containing yeast extract 0.4%, malt extract 1% and glucose was substituted with agricultural substrates i.e. palm kernel cake at 1%, 5% and 10% concentrations in 100 mL erlenmeyer flasks. All flasks were autoclaved at 121 °C for 20 min then cooled to room temperature. After cooling, media were inoculated with 10% of bacterial inoculum pre-grown for 3 days. Flasks containing inoculated media were incubated at room temperature on a rotary shaker at 120 rpm. Submerged fermentation was conducted for six days using a set of three flasks for each substrate used. Enzyme production was achieved under submerged fermentation; 3 mL culture was sampled at 1

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day intervals and the fermented matter was processed, as described above.

Phylogenetic analysis of strain ID06-379 based on 16S rDNA

DNA was isolated using PrepMan® Ultra Reagent (Applied Biosystems) according to the manufacturer's instruction. 16S rRNA gene was amplified by PCR using KOD FX (Toyobo) with the following pair of primers: 9F (5′-GAGTTTGATCCTGGCTCAG) and 1541R (5′-AAGGAGGTGATCCAGCC). The amplified 16S rRNA gene was subjected to cycle sequencing using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) with the following primers: 9F, 785F (5'-GGATTAGATACCCTGGTAGTC), 802R (5′-TACCAGGGTATCTAATCC) and 1541R. The products were analysed using an automated DNA sequencer (ABI PRISM 3730 Genetic Analyzer; Applied Biosystems).

The phylogenetic neighbours were identified and pairwise 16S rRNA gene sequence similarities were calculated, using the EzTaxon-e server (Kim *et al*., 2012). The CLUSTAL_X program (Thompson *et al*., 1997) was used to align the almost-complete 16S rRNA gene sequence of strain ID06-379 (1475 nt). Phylogenetic trees were reconstructed by the neighbor-joining (Saitou and Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) algorithms by using the MEGA 5.0 program (Tamura *et al*., 2011). The resultant tree topologies were evaluated by bootstrap analysis (Felsenstein, 1985) based on 1000 replicates.

Phenotypic characterisation of mannanolytic-actinomycetes

In order to determine colony appearance, strain ID06-379 was grown on yeast extract - malt extract (ISP-2) agar media at 30 °C for 7 days. Morphological features were observed by using a light microscope (BX-51; Olympus) and scanning electron microscope (JSM-5400; JEOL). The strain was grown on HV agar at 28 °C for 2 weeks; samples for SEM observation were prepared using the protocol of Tamura *et al.* (1994). Other physiological and biochemical tests were performed using API ZYM, API Coryne and API 50 CH systems (bioMérieux) according to the manufacturer's instructions.

Chemotaxonomic analysis

Cell material for the chemotaxonomic analyses was obtained by growing the strain in trypticase-soy broth (Difco) on a rotary shaker for 48 hand 100 rpm at 28 °C. The cells were harvested by centrifugation, washed twice with distilled water and freeze-dried. Amino acids and the isomers in cell-wall hydrolysates, cell-wall sugars, isoprenoid quinones were determined according to the methods described by Hamada *et al*. (2012) using LC mass spectroscopy. Polar lipids were extracted from 100 mg dry cells, purified using the method described by Minnikin *et al.* (1975) and analysed by TLC using chloroform/methanol/water (65 : 25 : 4, by vol.) in the first direction and chloroform/acetic acid/methanol/water (80 : 18 : 12 : 5, by vol.) in the second. For fatty acid methyl ester analysis, strain ID06-379 was cultured on tryptic soy agar (Difco) for 24 h at 28 °C. Cellular fatty acid methyl esters were analysed by GC (6890N; Agilent Technologies) according to the standard protocol of the Sherlock Microbial Identification System (Sasser, 1990) with Sherlock MIDI software (version 4.0) and a TSBA database (version 4.0). Genomic DNA for determination of the G+C content was prepared according to the method of Saito and Miura (1963) and was determined by enzymatic hydrolysis of DNA followed by reverse-phase HPLC, as described by Tamura *et al.* (1994).

RESULTS AND DISCUSSIONS

Selection of mannanase-producing actinomycetes

Based on the isolation sources, most of the isolates used in this study were isolated from soils. About 15% of tested isolates were able to degrade LBG, as indicated by the formation of a clear zone around the growing colonies, and measured as CZI (Figure 1).

From the 337 actinomycetes isolates screened, 49 isolates formed CZI between >1 to >5 on LBG media. Radial diffusion of enzyme into the substrate incorporated in an agar gel produced zones of hydrolysis which can be visualised by staining non-degraded substrate with Congo red dye (Carder, 1986). Colonies producing mannanase showed clear zones against red colour of non-hydrolysed medium.

Among the 49 isolates which showed high mannanase activity, most were isolated from soil at Kutai and Sungai Wain located in Kalimantan Island, which consists of tropical rainforests, and is marked by dense forests, valleys and rivers. A large area of the district is covered by green forests, which are rich in lignocellulosic materials derived from the thick vegetation of Dipterocarpaceae plants. Naturally, these fertile soils would have a rich assemblage of actinomycetes.

It has been observed that the environment of the soil, such as temperature, humidity, and diversity of the plants species grown on forest soil, influence the growth rate and diversity of microorganisms. The temperature between 27-29 °C and humidity around 66-88% at the sampling sites (Table 1) supports an abundant of microorganisms in that area. Tropical rainforests have abundant lignocellulosic material as a substrate for mannanolytic microorganisms. This condition is supposed to be an optimal condition for the growth of mannanolytic actinomycetes, taking part in the degradation of lignocellulosic material and responsible for the cycling of organic compounds. Soil microorganisms are vital for the continuing cycling of nutrients and for driving above-ground ecosystems (Ovreas, 2000).

Eight isolates which shown high mannanase activity (*Nonomuraea* sp. ID06-0379 and *Streptomyces* sp. ID07-0328, ID07-0332, ID07-0338, ID07-0365, ID07-0459, ID07-0463 and ID07-0496) were chosen for CZI screening test, which was about 3 - <5. A CZI higher than

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1.50 was considered to be a potential producer of mannanases (Florencio *et al*., 2012). The enzymatic index can be used as a simple and rapid methodology to select strains with the ability to produce enzymes. The reproducibility of the 8 isolates was conducted by re-analysing them using the same method. One isolate, *Nonomuraea*sp. ID06-379 was then chosen for indicate the study because it has high activity to degrade LBG substrate (CZI = 6.48). This is based on the selection by *Nonomuraea* sp. strain ID06-0379, a rare actinomycetes, which is capable of producing mannanase enzyme with sufficiently high activity. Strain ID6-0379 was obtained from soil in Kutai National Park, using sodium dodecyl sulphate-yeast extract medium (Widyastuti and Ando, 2009).

Enzyme production under submerged fermentation with various concentration of palm kernel cake (PKC) as substrate

The substrate concentration plays an important role in enzyme production and activity. In this study, we employed the palm kernel cake at various concentrations under submerged fermentation. Mannanase production from strain ID06-0379 in submerged fermentation using PKC as a substrate was increased by substrate concentration from 1, 5 and 10% from 1 to 5 day of incubation. The highest mannanase activity obtained was 4.40 U/mL at 5% PKC concentration (Figure 2) after 5 days of incubation; this might be due to the high concentration of substrate, which will influence the maximum medium component and oxygen transfer rate. The activity decreased after 5 days of incubation. These phenomena are due to the production of mannanase as a primary metabolite, which was produced by the degradation of carbon sources such as palm kernel cake for the development of biomass during the growth phase.

Bhoria *et al.* (2009) reported that *Streptomyces* sp. PG-08-3 isolated from sand produced high yield mannanase enzyme without using an expensive material for fermentation medium. The microbe achieved the mannanase activity of 15 U/mL when grown in minimal media containing 0.5% guar gum as a sole carbon source and a mannanase activity of 33 U/mL when grown in media containing soybean meals as a nitrogen source in submerge fermentation. Chuan *et al.* (2006) reported that β-mannanase produced by *B. subtilis* in a submerged fermentation system was recorded at 8 U/mL, while done in the SSF system it was recorded achieved as high as 230 U/g dry PKC. Azis *et al.* (2008) also reported that submerged fermentation of PKC using *Aspergillus niger* FTCC 5003 resulted in a maximum mannanase activity of 104 U/mL following 8 days of fermentation.

Mannanolytic bacteria *Geobacillus stearothermophilus* L-07 (Sumardi *et al*., 2005), was reported to produce extracellular β-1,4-mannanases with the highest β-mannanase activity (0.52 U/mL) in culture medium containing locust bean gum as the best for producing extracellular β-1,4-mannanases. Meryandini *et al.* (2008) also reported an Indonesian actinomycetes identified as *Streptomyces costarianus* 451-3 isolated from Kalimantan, which has mannanolytic activity in the media containing 0.5% locust bean gum with the highest activity as 0.179 nkat/mL, and 0.051 nkat/mL on the medium containing copra 0.5%. Yopi *et al.* (2006) reported that hydrolysis using *Streptomyces lipmanii* and *Kitasatospora* sp. that both isolates have the potential to produce mannanase with a mannanase activity of 0.032 U/mL and 0.133 U/mL, respectively.

This result showed that although mannanase enzyme produced from *Nonomuraea* sp. strain ID06-379 had a lower enzyme activity compared with mannanase produced from fungi, and compared with among other mannanolytic bacteria were reported, this enzyme have a good activity.

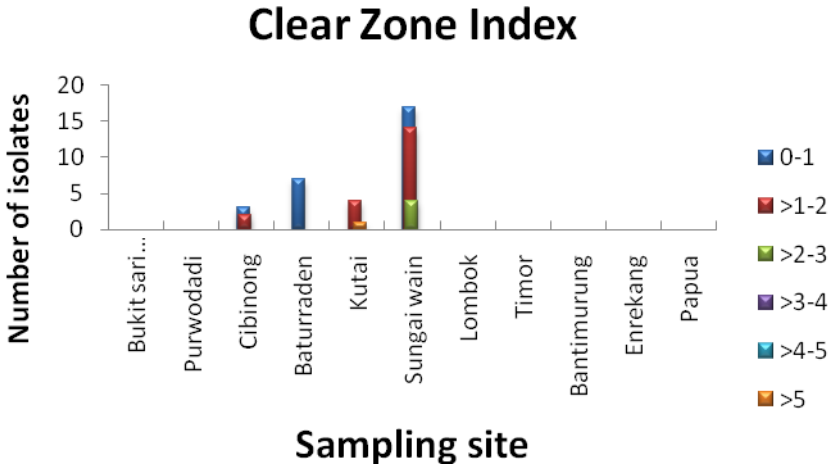

Figure 1: Index mannanolytic from screened actinomycetes.

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Table 1: Geographical position and regional climate characteristics.

Sampling site	Source		Latitude			Longitude			Altitude (m)	Temperature (°C)	Environment
	Soil	Leaf litter	E/W			N/S					
Purwodadi	1		E	112	44	S	07	48	290-300	31-32.4	Botanical garden
Bukit sari Jambi	1		E	102	47	S	01	34	60-70	30.7-34.3	Tropical forest
Cibinong	10		E	106	50	S	06	29	161-170	31.1-32	Dry land plantation
Enrekang	2		E	119	45	S	03	28	626-796	24-29	Burnt forest
Timor	7		E	123	49	S	10	15	25-562	24.7-34.7	Dry land savanna
Kutai	32		E	117	27	N	0	31	26-94	27.3-29	Tropical rainforest
Lombok	13		E	116	04	S	08	24	0.0-177	28-33.7	Dry land
Sungai Wain	83	41	E	116	51	S	01	08	40-50	28-30	Tropical rainforest
Baturraden	37	7	E	109	12	S	07	14	600-750	20-30	Pine forest
Bantimurung	85		E	119	45	S	04	54	ND	ND	Karst Cave ecosystem
Grasberg Papua	18		E	137	07-13	S	04	03-08	1900-4000	5-15	Highland mining area
Total	289	48									

Source: Widyastuti and Ando (2009) and Lisdiyanti *et al*. (2012).

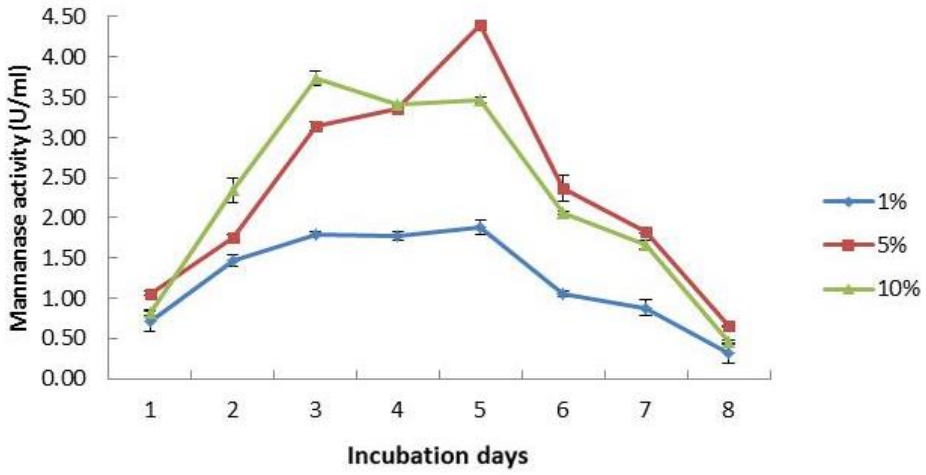

Figure 2: Mannanase activity of strain ID06-0379 on PKC substrate. Concentration of palm kernel cake 1% (▲triangles), 5% (■ squares) and 10% concentration (♦ diamonds) in in the medium for mannanase production contained 0.4% yeast extract, 1% malt extract, and 0.4% locust bean gum (pH 7.3). The culture was incubated at room temperature in a rotary shaker at 120 rpm.

Phylogenetic analysis based on 16S rDNA

Comparative analysis of 16S rDNA sequences and phylogenetic relationships showed that strain ID06-379 grouped in a subclade with *Nonomuraea jabiensis* A4036T, supported by a bootstrap value of 68% in the neighbour joining analysis (Figure 3), with which it shared a 16S rDNA sequence similarity of 99%, this value corresponds to 6 nt differences at 1475 locations. 16S rDNA nucleotide similarities values were ranging from 97% (*N. soli* strain YIM 120770) to 99% (*N. jabiensis* A4036^T) have been recorded for members of several validly described *Nonomuraea* type strain species. Phylogenetic analysis based on 16S rDNA sequence revealed that a closely related species for *Nonomuraea* sp. ID06-0379 was *N. jabiensis* A4036T.

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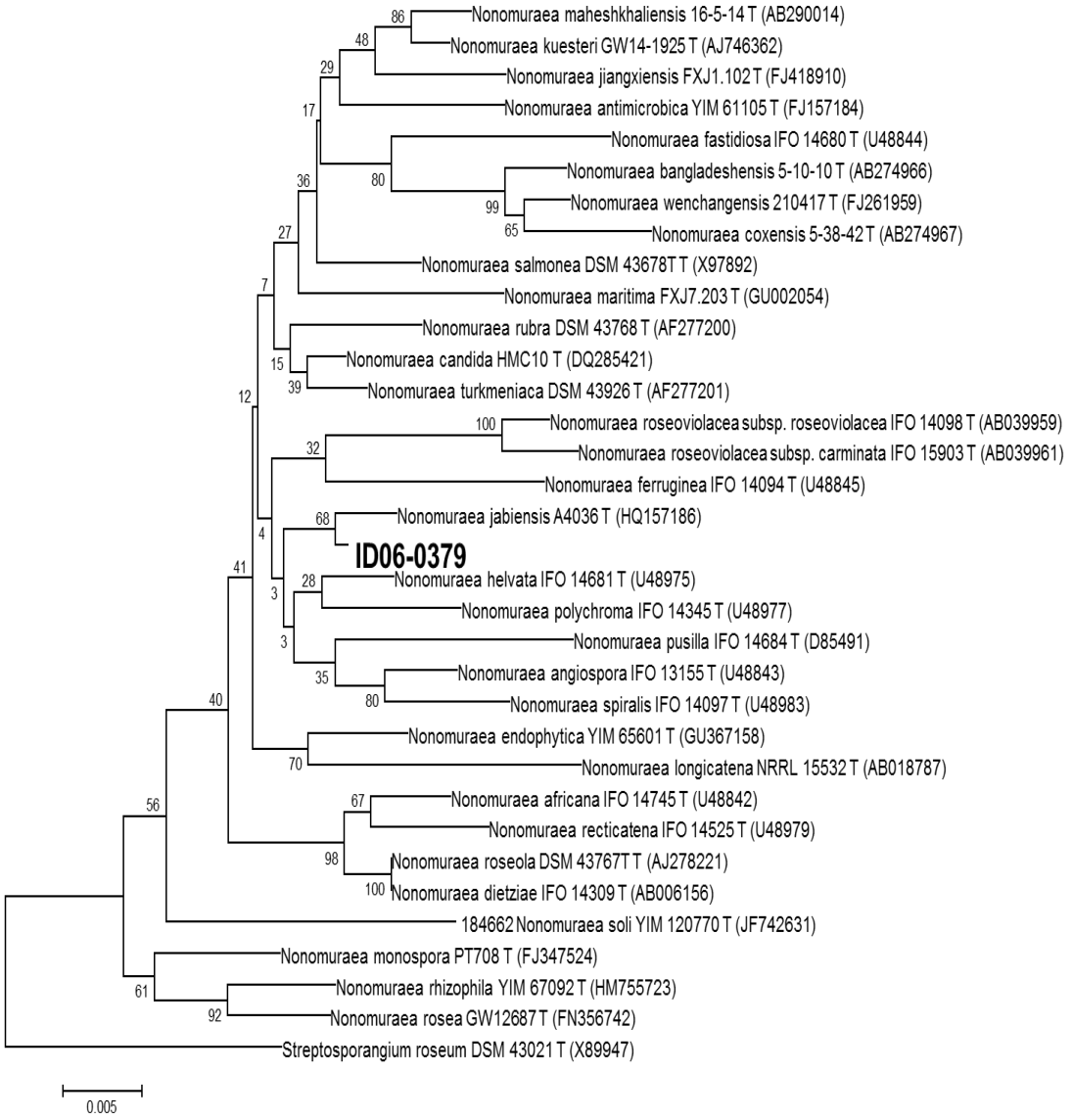

Figure 3: Neighbour-joining tree based on 16 rDNA sequences (1475 nt) showing the position of strain ID06-0379 amongst its phylogenetic neighbours. *Streptosporangium roseum* DSM 43021^T was used as an outgroup. The tree was constructed using the neighbour-joining method and K_{nuc} values.

Phenotypic characteristics of mannanolytic-actinomycetes strain ID06-0379

Orange colour colonies appeared in the early stages of growth and became white orange in colour during the incubation period. Variability in the result of colony colour could be due to differences in colony age, variations in soil habitat and vegetation that could affect the species composition and their morphological and physiological characteristics (Saadoun *et al*., 2007). According to Lechevalier and Lechevalier (1980), the colour that appears in the actinomycetes colony result from pigmentation.Colony of strain ID06-0379 produced a nearly round shape, rugous edges, and a surface that was hairy. The strain forms extensively branched, brownish orange substrate mycelia that bear white aerial hyphae on ISP 2. After 14 days of incubation at 28 °C, mycelium grown from vegetative branches formed mycelia fibres (Figure 4a); the strain formed extensively branched substrate mycelia and aerial hyphae that often formed spiral chains of spores with warty surfaces (Figure 4b-d), and spiral spore chains composed of warty surface spores were observed on the aerial mycelium (Figure 4d).

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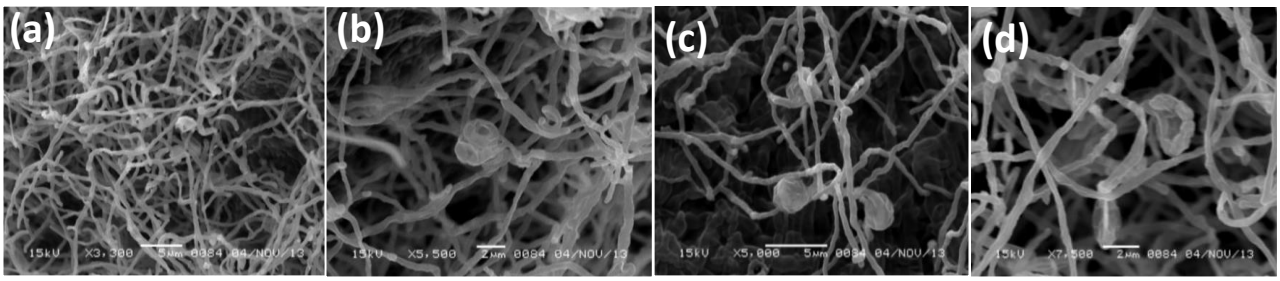

Figure 4: Scanning electron micrographs of *Nonomuraea* sp. strain ID06-0379 grown on HV agar at 28 °C for 2 weeks. The hooked/ curled spores chain morphology is clearly visible in (a) and (b). Spiral spores chains covered with sheath on aerial mycelium tips (c). Curled spores chains with warty surface (d). The size bar 2 µm (b and d), 5 µm (a and c).

Chemotaxonomic characterisation of strain ID06-379

The whole-cell hydrolysate of the strain ID06-0379 contained *meso*-diaminopimelic acid (cell wall type III Lechevalier and Lechevalier, 1970) as the diagnostic diamino acid in the cell-wall peptidoglycan, and the whole-cell sugars had mannose, glucose, ribose (major components), and madurose (Type B; madurose as a diagnostic sugar), with galactose as the minor component. The polar lipids of strain ID06-379 are of the PIV type, according to the phospholipid classification of Lechevalier *et al.* (1977), it were phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG), phosphatidylinositol (PI), and hydroxy-phosphatidylethanolamine (OH-PE). Many members of the genus *Nonomuraea* have been shown to produce PE and OH-PE (Kämpfer *et al.*, 2010). OH-PE has been found in *N.longicatena* (Chiba *et al*., 1999), *N. bangladeshensis*, *N. coxensis* (Ara *et al*., 2007a), and *N. maheshkhaliensis* (Ara *et al*., 2007b). This polar lipid profile is generally similar to those of recognized species of the genus *Nonomuraea*.

The quinone system found supports affiliation of strain ID06-379 to the genus *Nonomuraea.*The results of LC-mass spectroscopy, demonstrate that strain sp. ID06-379 contained the menaquinone identified as MK-9. The predominant menaquinone of strain ID06-0379 was MK-9(H4) (59.55%); MK-9(H2) (24.3%), MK-9(H0) (13.6%) and MK-9(H6) (2.5%) were also detected. This is essentially in accordance with the quinone profile reported for members of the genus (Quintana *et al*., 2003). MK-9 is the major menaquinone component in many Gram-positive bacteria (Das *et al*., 1989) and was suggested to be the major menaquinone in the family Streptosporangiaceae.

The major cellular fatty acids of the strain ID06-379 were $C_{16:0}$ (31.47%), cis9-$C_{16:1}$ (15.23%) and iso-$C_{16:0}$ (10.84%); minor amounts of cis9-$C_{18:1}$ (7.03%), cis9-$C_{17:1}$ (6.75%), C14:0 (6.47%), 10-methyl-$C_{16:0}$ (5.79%), $C_{15:0}$ (3.11%), 10-methyl-$C_{17:0}$ (2.83%), $C_{17:0}$ (2.41%), iso-$C_{15:0}$ (2.32%) and were also present. The G+C content of the DNA was 71.7 mol%. The chemical and morphological properties of strain ID06-379 are consistent with its classification in the genus *Nonomuraea* (Tamura *et al*., 2000).

This results showed that on the bases of chemotaxonomic characterization, 16S rDNA sequence and phylogenetic tree analyses of strain ID06-379 was revealed that this characteristics are similar with the characteristics shown by the closest species *N. jabiensis* A4036T (Camas *et al*., 2013).

The differences in physiological characteristics between strain ID06-0379 with those of species of the genus *Nonomuraea* with validly published names are presented in Table 2. There were slightly physiological and biochemical features that differed between strain ID06-379 and *N. jabiensis* A4036^T, such as differences in nitrate reduction, degradation of gelatin, starch, urea, and utilization of sole carbon sources. Strain ID06-379 and *N. jabiensis* A4036^T have positive result in test for arbutin, and aesculin hydrolysis (Camas *et al*., 2013), but strain ID06-379 has negative result for urea hydrolysis and nitrate reduction. D-galactose, D-fructose, D-mannose, D-mannitol, arbutin, D-cellobiose, D-maltose, D-sucrose are used as sole carbon source, but D-xylose, maltose, L-xylose, L-sorbose, inulin and D-melezitose are not. This result also supported that strain ID06-0379 is belongs to species in the cluster of *Nonomuraea* species.

This study was the first report a new information that the species of *Nonomuraea* produced mannanase enzyme. It may need optimised media in enzyme production by using carbon or nitrogen sources due to different requirements by the isolate. Actinomycetes possess the potential to secrete a broad range of enzymes, which may be the results of natural selection of the microorganisms in order to survive in a competing environment (Jeffrey, 2008).

Mannanase enzyme produced by *Nonomuraea* sp. ID06-379 showed high activity, similar to that for *Streptomyces costarianus* 451-3, *S. lipmanii* and *Kitasatospora* sp. (Meryandini *et al*., 2008; Yopi *et al*., 2006) or other bacteria (Sumardi *et al*., 2005) but lower than the enzyme production of fungal mannanase from *A. niger* FTCC 5003 (Azis *et al*., 2008). This result showed that strain *Nonomuraea* sp. ID06-0379 has the potential to produce bacterial enzyme mannanase using agricultural waste such as palm kernel cake.

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Table 2: Phenotypic properties that differentiate strain ID06-0379 with those of species of the genus *Nonomuraea* with validly published names. Species: 1. ID06-379, 2. *N. jabiensis* A4036^T, 3. *N. angiospora* DSM 43173^T, 4. *N. candida* DSM 45086^T, *5. N. spiralis* DSM 43555^T, 6. *N. roseoviolacea* subsp. *roseoviolacea* DSM 43144^T, 7. *N. pusilla* DSM 43357^T.

Biochemical test	1	2	3	4	5	6	7
Nitrate reduction	-	+	-	+	+	+	+
Degradation of							
Gelatin hydrolysis	-	+	+	-	+	-	-
Starch	w	-	+	-	-	-	-
Growth on sole carbon source							
D-Xylose	-	+	+	+	+	+	-
Maltose	-	+	+	+	-	+	-
Sucrose	-	+	+	-	+	+	-
L-Xylose	-	+	+	+	+	+	-
D-Adonitol	w	+	+	-	-	+	+
D-Galactose	w	-	+	+	-	-	-
D-Fructose	w	+	+	+	-	+	-
D-Mannose	+	+	+	+	+	-	-
L-Sorbose	-	-	+	-	-	-	-
D-Mannitol	w	+	+	+	-	+	-
Arbutin	+	-	+	+	-	+	+
D-Cellobiose	w	-	-	+	-	+	+
D-Maltose	w	+	+	+	-	+	-
D-Sucrose	+	+	+	+	+	+	-
Inulin	-	+	+	+	-	+	-
D-Melezitose	-	+	-	-	+	+	-

Data are taken from Camas *et al.* (2013), Chiba *et al*. (1999), and Quintana *et al*. (2003). Symbols: +, positive; -, negative; W, weak.

CONCLUSION

From the 337 actinomycetes isolates screened, about 15% were capable of degrading LBG, as indicated by the formation of a clear zone around the growing colonies, which was measured as clear zone index (CZI). Phylogenetic analysis of 16S rDNA sequence revealed that a closely related species for the strain ID06-0379 was *N. jabiensis* A4036^T, which has 99% nucleotide similarity. On the basis of phenotypic and chemotaxonomic characters of the strain ID06-379, it was concluded that the strain was placed in the cluster of *Nonomuraea* species. This study was the first to report new information that the species of *Nonomuraea* produced mannanase enzyme; the best mannanase activity obtained was 4.40 U/mL at 5% PKC concentration. These findings indicate that *Nonomuraea* sp. ID06-0379 was a potential isolate for the production of the bacterial enzyme mannanase using agricultural waste such as palm kernel cake and may contribute to the development and utilisation of biomass bioconversion processes.

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